Riggs, A. D., Suzuki, H., & Bourgeois, S. (1970) *J. Mol. Biol. 48*, 67–83.
Saxe, S. A., & Revzin, A. (1979) *Biochemistry 18*, 255–263.
Senear, A. W., & Steitz, J. A. (1976) *J. Biol. Chem. 251*, 1902–1912.
Spierer, P., Bogdanov, A. A., & Zimmerman, R. A. (1978) *Biochemistry 17*, 5394–5398.
Usher, D. A., & Rosen, J. A. (1979) *Anal. Biochem. 92*, 276–279.
Walker, G. C., Uhlenbeck, O. C., Bedows, E., & Gumport, R. I. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 122–126.
Wells, R. D., Hardies, S. C., Horn, G. T., Klein, B., Larson, J. E., Neuendorf, S. K., Panayotatos, N., Patient, R. K., & Selsing, E. (1980) *Methods Enzymol. 65*, 327–347.

Interaction of *Escherichia coli* Host Factor Protein with Qβ Ribonucleic Acid†

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ABSTRACT: The affinity of *Escherichia coli* host factor protein for a variety of ribonucleic acids (RNAs) is compared in an equilibrium competition assay with $(pA)_{15}$ or $(pA)_{27}$ as the common probe. Of the homopolymers tested, only polyriboadenylate [poly(rA)] binds the protein with a high affinity. At low ionic strength (0.1 M NaCl), the binding to Qβ RNA is much stronger than to the oligoadenylates, but the situation is reversed upon fragmentation of the RNA with ribonuclease T_1. Increasing the ionic strength results in a drastic reduction of the affinity of host factor for Qβ RNA over a relatively narrow salt range (0.1–0.3 M NaCl). Over the same range, added salt greatly reduces the tendency of host factor hexamers to aggregate. The tight binding of host factor to Qβ RNA is proposed to result from the binding of an aggregate, which can interact with several low affinity sites on the RNA simultaneously.

In the previous paper (de Haseth & Uhlenbeck, 1980) the RNA[1] binding site of host factor was characterized by studying the binding properties of oligo(A)'s. Each host factor hexamer can accommodate 16–18 contiguous nucleotides in a circular orientation. The interaction between host factor and $(pA)_{18}$ is extremely strong, with a binding constant of $>3 \times 10^{10}$ M^{-1} in 0.1 M NaCl. Only a small portion of the free energy is due to ionic interactions, since the association constant is only weakly dependent on the salt concentration of the binding buffer. The relationship of the function of host factor to its RNA binding properties is of considerable interest (Blumenthal & Carmichael, 1979). In its only known function, host factor greatly stimulates the synthesis of RNA by Qβ replicase in vitro, apparently by binding the Qβ RNA plus strand at a limited number of sites (Franze de Fernandez et al., 1972). In a more detailed study of the host factor–Qβ RNA interaction, Senear & Steitz (1976) were able to isolate two ribonuclease T_1 digest fragments of Qβ RNA by their retention with host factor on nitrocellulose filters. From a comparison of their sequence and the sequence of a similarly retained T_1 fragment of R_{17} RNA, Senear & Steitz (1976) suggested that host factor bound specifically to the sequence AAUAAA or a variant thereof. It appeared reasonable that host factor would bind to the same sequence in intact Qβ RNA as well. Since the position of these two sequences was internal in the Qβ RNA molecule, their relation to the function of host factor in the stimulation of the initiation of RNA synthesis by Qβ replicase remained unclear.

We report here studies on the interaction of host factor with various homopolymers and natural RNAs. A major goal is to determine the degree to which the interaction of host factor with Qβ RNA is a specific one. The relative ability of various RNAs to compete with a $[5'\text{-}^{32}P](pA)_{27}$ probe for host factor binding, as measured by millipore filter retention, is the primary assay used in this work. By use of a common, well-characterized probe to compare different RNAs, possible complications of variable backgrounds or retention efficiencies of the different RNAs are circumvented. Our results show that intact Qβ RNA binds host factor with a much higher affinity than oligo(A), but the T_1 digest of Qβ RNA binds much less well than oligo(A). Thus the T_1 fragments are not solely responsible for the tight binding of host factor to Qβ RNA. We suggest that the aggregation of host factor hexamers, which we observe at low ionic strength, is involved in the tight binding of host factor to Qβ RNA.

Materials and Methods

RNAs. Poly(A) and poly(C) were from Sigma Chemical Co., poly(U) and poly(I) were from Miles Laboratories, and poly(dA) was from P-L Biochemicals. In each case more than 80% of the material was larger than tRNA, as determined by electrophoresis on denaturing 20% acrylamide gels. Residue extinction coefficients were from Janik (1971). [8-^{3}H]Poly(A) (60 Ci/mol) was synthesized from [8-^{3}H]ADP (Amersham) by using *Micrococcus luteus* polynucleotide phosphorylase. From its migration on denaturing gels a mean chain length of 160 ± 50 nucleotides was estimated.

Qβ RNA was obtained as gifts from P. Cole, C. Guerrier-Takada and K. Campbell. The RNA was isolated from purified phage and further purified on sucrose gradients. STNV RNA was a gift from K. Browning and J. Clark, and

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[1] Abbreviations used: RNA, ribonucleic acid; poly(A) polyadenylate; poly(C), polycytidylate; poly(U), polyuridylate; poly(I), polyinosinate; poly(dA), polydeoxyadenylate; tRNA, transfer RNA; oligo(A), oligoadenylate; Tris, 2-amino-(2-hydroxymethyl)-1,3-propanediol; EDTA, ethylenediaminetetraacetic acid; BSA, bovine serum albumin; DNA, deoxyribonucleic acid; rRNA, ribosomal RNA.

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Escherichia coli 16S rRNA was a gift from C. Cantor. The residue extinction coefficient of these RNAs was assumed to be 7×10^3 M^{-1} cm^{-1}. The following values were used for the molecular weights of the RNAs: Qβ RNA, 1.5×10^6 (Boedtker & Gesteland, 1975); 16S rRNA, 5.6×10^5 (Kurland, 1960); STNV RNA, 4×10^5 (Reichmann, 1964).

RNase T_1 digestions were carried out as described by Senear & Steitz (1976). RNA and RNase T_1 (Calbiochem) at a 20:1 weight ratio were incubated at 37 °C in 10 mM Tris, pH 7.5, 0.1 M NaCl, and 0.1 mM EDTA. After 30 min the mixture was chilled on ice. It was assayed for host factor binding activity within 20 min without further purification.

Filter binding assays were carried out as described in the preceding paper (de Haseth & Uhlenbeck, 1980), in a buffer containing 10 mM Tris, pH 7.5, 1 mM EDTA, 3.5 mM β-mercaptoethanol, 10 μg/mL BSA, and NaCl to the concentration indicated. At 1.0 M NaCl, solutions containing [^{3}H]poly(A) had a very high background retention in the absence of host factor. In this case only, the filters were rinsed with 5 mL of 0.1 M NaCl buffer after filtration. The details of the competition assay for detecting host factor binding, the host factor preparation used, and the preparation of [5′-^{32}P]oligo(A)'s are described in the preceding paper. The assay mixtures contained, in buffer, 0.76 nM host factor hexamers, 0.83 nM or more [5′-^{32}P]oligo(A) (~5×10^4 cpm), and competing RNA as indicated. After incubation for 15 min at 23 ± 2 °C, the mixtures were filtered and processed as before. Experiments were carried out at several concentrations of competing RNA to allow the determination of $[R_t]_{0.5}$, the concentration of competing RNA needed to give a 50% reduction in the amount of [^{32}P]oligo(A) retained on the filter. As described in the preceding paper (de Haseth & Uhlenbeck, 1980), there is an inverse relationship between $[R_t]_{0.5}$ and the ability of a particular RNA to compete in the assay. This, in turn, is a function of both the stoichiometry of the interaction and the binding constant to each site. In the absence of independent information these cannot be resolved. With some competitors, even the highest amount of RNA added resulted in a <10% reduction in the amount of probe retained. In these cases a lower limit of $[R_t]_{0.5}$ is given as 10 times the highest amount of RNA added, based on the empirical functional form of the competition curve (de Haseth & Uhlenbeck, 1980).

Gel filtration chromatography of host factor in the presence and absence of [^{32}P]oligo(A)'s was performed on columns packed with Sephadex G-200 (Pharmacia Fine Chemicals). The columns (1.35 × 80 cm) were run in 10 mM Tris, pH 7.5, 1 mM EDTA, 3.5 mM β-mercaptoethanol, and NaCl as indicated. The flow rate was 10 mL/h and 1.7-mL fractions were collected. For runs at room temperature (23 ± 2 °C) the recovery of material off the column was low but could be improved by the inclusion of 150 μg of poly(C) and 50 μg of BSA to the 1-mL sample typically loaded on to the column. Aldolase (200 μg; Sigma Chemical Co.; 160 000 daltons) was included as a molecular weight marker.

Results

(*a*) *Interaction of Host Factor with Homopolymers.* The relative affinities of a variety of homopolymers and RNAs for host factor can conveniently be compared by competing several concentrations of each one with a constant amount of [5′-^{32}P](pA)$_{27}$ for a constant amount of host factor. The protein is expected to show a generalized, or nonspecific, binding to overlapping sites distributed over the length of the polymer chains. For polymers which are long compared to the amount of RNA covered by the protein (as is the case here), the concentration of sites is determined by the concentration of *residues* in the polymers (McGhee & von Hippel, 1974). In addition, the chain lengths of the polymers are not accurately known. Thus, the calculation of molar association constants is neither desirable nor possible. Instead, the relative affinities can be compared by determining $[R_t]_{0.5}$, the concentration of competitor, expressed in moles of residue per liter, needed to reduce the binding of the (pA)$_{27}$ probe to half its uncompeted value. These data are given in Table I. The strong affinity of riboadenylylate residues for host factor (Carmichael, 1975) is confirmed by these data. No detectable competition with poly(C) or poly(dA) and only very weak competition with poly(I) were observed. Attempts to detect a complex with poly(C) and poly(dA) by quenching of host factor fluorescence at much higher concentrations were unsuccessful (data not shown). A weak association between poly(U) and host factor could be observed by fluorescence that could not be detected by (pA)$_{27}$ competition, presumably due to double-helix formation. Mixtures of poly(A) and poly(U) also showed little affinity for host factor, as detected in the fluorescence quenching and competition assays. These data confirm and extend the data of Carmichael (1975) who showed corresponding poor retention of host factor on poly(C)–, poly(I)–, and poly(U)–cellulose columns and Franze de Fernandez et al. (1972) who showed poor affinity of host factor for DNA. The results indicate that the high specific affinity of host factor for poly(A) must result from contacts with the ribose 2′-hydroxyl and one or more substituents on the adenine ring. Little of the total free energy of binding is due to ionic interactions with the phosphates (de Haseth & Uhlenbeck, 1980).

Table I: Interaction of Host Factor with Homopolymers

competitor[a]	[NaCl] (M)	$[R_t]_{0.5}$ (mol of residues/L)
(pA)$_{27}$	0.1	4.3×10^{-8}
	1.0	5.0×10^{-8}
poly(A)	0.1	2.1×10^{-8}
	1.0	7.7×10^{-8}
poly(dA)	0.1	$>5 \times 10^{-6}$
	1.0	$>2.5 \times 10^{-6}$
poly(C)	0.1	$>2.5 \times 10^{-6}$
poly(I)	0.1	1.3×10^{-6}

[a] Probe, 1.5 nM [5′-^{32}P](pA)$_{27}$.

A comparison of the competition behavior of (pA)$_{27}$ and poly(A) shows that while at 0.1 M NaCl poly(A) is a slightly better competitor than (pA)$_{27}$ per mole of A residues added, the situation is reversed at 1.0 M NaCl. This behavior is not fully understood. Plausible explanations will have to take into account not only the fact that end effects are relatively more important for (pA)$_{27}$ than for poly(A), but also the aggregation properties of host factor (see below). Since host factor binds (pA)$_{27}$ with a 1:1 stoichiometry under these conditions (de Haseth & Uhlenbeck, 1980), the binding density of host factor hexamers on the poly(A) at 50% competition can be estimated if the concentration of free host factor is neglected. Thus it is calculated that one hexamer is bound per 60 residues of poly(A) in 0.1 M NaCl and one per 210 residues of poly(A) in 1.0 M NaCl. Under conditions of close packing one hexamer covers 14–18 residues (de Haseth & Uhlenbeck, 1980). A strong cooperative binding of host factor to poly(A), analogous to the interaction of T4 gene 32 protein (Alberts & Frey, 1970) or *E. coli* helix–destabilizing protein (Ruyechan & Wetmur, 1975) with single–stranded DNA, appears therefore not likely.

(*b*) *Interaction of Host Factor with Natural RNAs.* The

Table II: Interaction of Host Factor with Natural RNAs in 0.1 M NaCl

competitor[a]	$[R_t]_{0.5}$ (nmol of residues/L)	$[R_t]_{0.5}$ (pmol of RNA molecules/L)
Qβ RNA	230	50
STNV RNA	230	200
16S rRNA	160	100

[a] Probe, 0.83 nM [5′-^{32}P](pA)$_{27}$.

Table III: Effect of Concentration of Probe on Competition by Qβ RNA in 0.1 M NaCl

$[(pA)_{27}]$ (nM)	$[R_t]_{0.5}$ (pmol of RNA molecules/L)
0.83	51 (3)[a]
1.50	33
1.66	34 (2)
7.50	56

[a] Parentheses indicate the number of independent determinations.

results of competition experiments between $(pA)_{27}$ and Qβ RNA, 16S RNA, or STNV RNA for host factor binding are given in Table II. The concentrations of the three natural RNAs (in moles of residue per liter) required to reach 50% competition at 0.1 M NaCl are similar. Much larger amounts of these RNAs are required however than was the case with poly(A) (compare Table I). This difference is not due to a lower binding constant for the natural RNAs but is a consequence of a very different stoichiometry of binding in the two cases. In the case of poly(A), each contiguous sequence of 16 residues in the polymer forms a binding site for host factor (de Haseth & Uhlenbeck, 1980), whereas the natural RNAs have only a few, very strong binding sites. Since the stoichiometry of host factor binding to the $(pA)_{27}$ probe was shown to be equimolar and negligible amounts of free host factor are present in the competition experiments, one can calculate the number of host factor hexamers bound to each RNA, at the point of 50% competition, by using the values in the final column of Table II. Thus each Qβ RNA has eight hexamers bound to it (over the course of several experiments a range of six to twelve hexamers per Qβ RNA was found), while four and two hexamers are bound to the shorter 16S and STNV RNA molecules, respectively. The presence of a few high-affinity host factor binding sites on Qβ RNA is confirmed by the data in Table III. In this case similar competition experiments between Qβ RNA and $(pA)_{27}$ for host factor were carried out at several concentrations of the $(pA)_{27}$ probe. An almost 10-fold increase in the concentration of $(pA)_{27}$ did not result in a significant increase in the amount of Qβ RNA necessary to get 50% competition. Thus, the binding constant of host factor to its sites on Qβ RNA is, at 0.1 M NaCl, at least 10 times higher than the binding constant for the host factor $(pA)_{27}$ interaction. At this salt concentration, a value of 1.6×10^{11} M^{-1} was found for the binding constant of the host factor–$(pA)_{27}$ interaction (de Haseth & Uhlenbeck, 1980). Thus, an effective binding constant of at least 10^{12} M^{-1} is indicated for each of the host factor hexamers bound to Qβ RNA.

The value of six to twelve host factor hexamers tightly bound per Qβ RNA obtained here is somewhat higher than that determined by others using different approaches. Senear & Steitz (1976) measured the retention of ^{32}P-labeled Qβ RNA by host factor on nitrocellulose filters and obtained maximal retention with approximately three hexamers per RNA molecule (recalculated from their Figure 1A). However, since filter-retention experiments only determine the minimal amount of bound protein which will cause retention of the RNA to the filter, this experiment does not determine the total number of sites on Qβ RNA and thus is not inconsistent with the estimate here. Franze de Fernandez et al. (1972) and Carmichael et al. (1975) found maximal stimulation of Qβ RNA directed Qβ replicase activity by host factor at a molar ratio of about two hexamers per RNA. Our results can be reconciled with these by simply assuming that only a few hexamers, binding tightest among the six to twelve reported here, are required for stimulation of initiation of RNA synthesis.

Table IV: Effect of Digestion with RNase T_1 on Competition by Qβ RNA in 0.1 M NaCl

probe	competitor	$[R_t]_{0.5}$ (mol of RNA molecules/L)
[5′-^{32}P](pA)$_{15}$, 1.5 nM	Qβ RNA	1.4×10^{-11}
	Qβ RNA + T_1[a]	6.2×10^{-9} [b]
	$(pA)_{15}$	1.7×10^{-9}
[5′-^{32}P](pA)$_{27}$, 0.83 nM	Qβ RNA	3.0×10^{-11}
	Qβ RNA + T_1[a]	$>1.3 \times 10^{-8}$ [b]
	$(pA)_{15}$	1.1×10^{-8}
	$(pA)_{15}$ + 30× T_1[c]	1.3×10^{-8}
	$(pA)_{27}$[d]	1.2×10^{-9}

[a] Qβ RNA incubated with RNase T_1 (see text). [b] Concentration of Qβ RNA equivalents. [c] $(pA)_{15}$ incubated with a 30× higher weight ratio of T_1 than used for Qβ RNA. [d] From de Haseth & Uhlenbeck (1980).

A possible explanation for the high affinity of host factor for Qβ RNA is that the site (or sites) on Qβ RNA has a sequence to which host factor binds with a particularly high binding constant. This view was suggested by Senear & Steitz (1976) after successful selection of two A-rich oligo nucleotides from a T_1 digest of Qβ RNA, by filtration through a nitrocellulose filter in the presence of host factor. These sequences would be expected to have a binding constant higher than oligo(A) and thereby account for the high affinity of Qβ RNA for host factor. In Table IV this possibility is tested by comparing the ability of Qβ RNA and a total T_1 digest of Qβ RNA to compete with two oligo(A)'s for host factor binding. Chain lengths of 15 and 27 residues were chosen, since they differed considerably in their binding constants (de Haseth & Uhlenbeck, 1980) and were close to the chain lengths of the T_1 oligonucleotides retained in the selection experiments (15 and 26 nucleotides). The results of these competition experiments indicate that the T_1 oligonucleotides bind *less* well than the corresponding oligo(A)'s which in turn bind less well than Qβ RNA. Treatment of 16S rRNA and STNV RNA with RNase T_1 also practically abolished their ability to compete with the $(pA)_{27}$ probe. In a control experiment, proper selection of the larger T_1 oligomer of Qβ RNA from a mixture of 5′-^{32}P-labeled T_1 oligonucleotides by host factor was observed (data not shown). Since the Qβ T_1 oligomers bind at least 3 orders of magnitude less well than Qβ RNA, it is clear that their sequence alone cannot be responsible for the high affinity of Qβ RNA for host factor. Although other, T_1-sensitive, high-affinity binding sites for host factor may exist on Qβ RNA, it is important to look for alternative possibilities as well.

(*c*) *Ionic Strength Dependence of RNA–Host Factor Interactions.* Competition experiments between $(pA)_{27}$ probe and varying amounts of Qβ RNA and STNV RNA for host factor were carried out at several ionic strengths, and a $[R_t]_{0.5}$ was determined in each case. Since the binding constant for

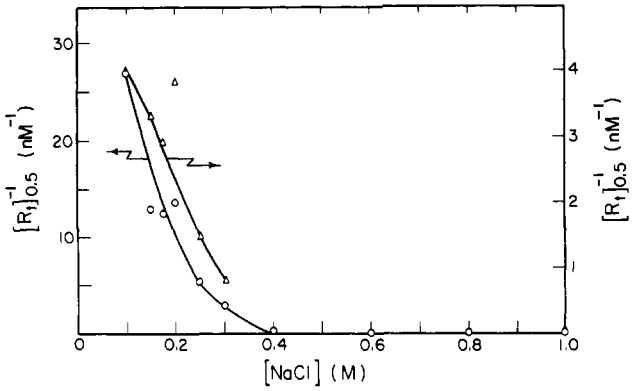

FIGURE 1: Ionic strength dependence of competition by either Qβ RNA (○) or STNV RNA (△) for [5′-^{32}P](pA)$_{27}$. 1-mL buffered reactions at indicated ionic strength contained 0.76 nM host factor hexamer, 0.83 nM [5′-^{32}P](pA)$_{27}$, and varying amounts competitor RNA. From each such competition, a value of $[R_t]_{0.5}$ was obtained.

(pA)$_{27}$ decreases by a factor of ~20 between 0.1 and 1.0 M NaCl (de Haseth & Uhlenbeck, 1980), this experimental design focuses on how the two RNAs differ from (pA)$_{27}$ in their sensitivity to ionic strength when binding to host factor. In Figure 1, $[R_t]^{-1}_{0.5}$, which is proportional with the ability of the RNAs to compete for host factor binding, is shown as a function of ionic strength. It is seen that the two RNAs both become much worse competitors as the ionic strength is increased. At 0.3 M NaCl 10 times as much Qβ RNA is required to reach 50% competition than was needed at 0.1 M NaCl. At 1.0 M NaCl we were not able to demonstrate *any* competition between Qβ RNA and (pA)$_{27}$ even when 30 times as much Qβ RNA was added than would give 50% competition at 0.1 M NaCl. This sets a lower limit for $[R_t]_{0.5}$ of 10^{-8} M for Qβ RNA at 1.0 M NaCl. STNV RNA and 16 S rRNA also failed to show any detectable competition at 1.0 M NaCl. This behavior is to be contrasted to poly(A) which only shows a slightly higher $[R_t]_{0.5}$ at 1.0 M NaCl than at 0.1 M NaCl (Table I). From the data in Figure 1, it is estimated that at 1.0 M NaCl host factor binds to Qβ RNA with an association constant at least 100 times *less* than that for the interaction with (pA)$_{27}$, while at 0.1 M NaCl it was found that Qβ RNA binds host factor with an association constant at least 10 times *greater* than (pA)$_{27}$. The fact that the affinity of host factor for Qβ RNA is much more salt sensitive than its affinity for (pA)$_{27}$ opens up the possibility that the natures of the two complexes are quite different from one another.

Another indication that the nature of the host factor–RNA interaction changes considerably with ionic strength can be seen in the titration of a constant amount of [^{3}H]poly(A) with host factor as detected by filter binding in Figure 2. More moles of host factor were needed to retain the poly(A) on the filter at low ionic strength than at high ionic strength. This would apparently contradict the fact that the association constant of poly(A) to host factor is actually slightly higher at low ionic strength (Table I). This anomaly is not explained by differences in the retention efficiency of complexes on the filter since the plateau of maximal retention is not a function of ionic strength. The simplest explanation for the change in the stoichiometry of binding with ionic strength seen in Figure 2 is that host factor hexamers aggregate at low ionic strength while retaining their ability to bind poly(A). An estimate of the extent of aggregation can be obtained from the initial slope of the titrations in Figure 2 where it may be assumed that each poly(A) molecule (average length, 160 ± 50 residues) only has the minimal amount of protein attached to it to bind the complex to the filter. We calculate that an average of one to

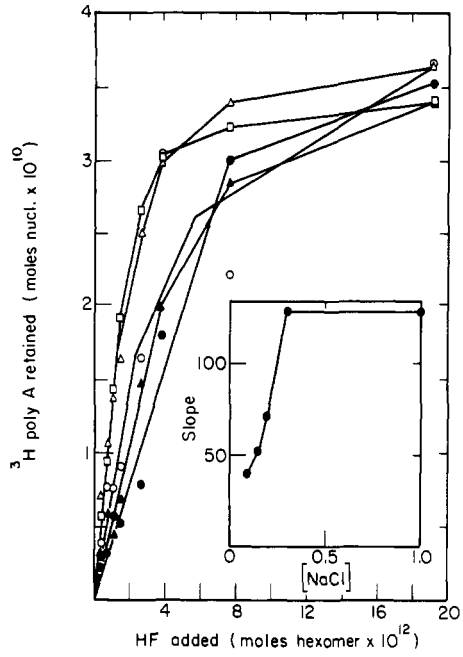

FIGURE 2: Retention of [^{3}H]poly(A) as a function of NaCl concentration. Mixtures contained 0.37 nmol of nucleotide of poly(A), varying amounts of host factor stock solution in 0.1 M (●), 0.15 M (▲), 0.2 M (○), 0.3 M (△), and 1.0 M (□) NaCl. (Insert) Slopes of lines, drawn through points below 50% maximal retention, vs. [NaCl].

two host factor hexamers are bound to each poly(A) molecule at 0.3 and 1.0 M NaCl and three to six hexamers are bound at 0.1 M NaCl. It would appear possible that these salt-dependent aggregation properties of host factor seen with poly(A) binding are also the explanation for the unusual ionic strength dependence of host factor binding to Qβ RNA.

(*d*) *Aggregation of Host Factor.* The aggregation of host factor hexamers as a function of ionic strength was examined directly by analytic Sephadex G-200 gel filtration chromatography. At low ionic strengths the apparent molecular weight of free host factor is indeed considerably higher than that of a hexamer. For example, in 0.1 M NaCl (Figure 3a) free host factor migrates ahead of aldolase (molecular weight 160 000) with an apparent molecular weight of ~210 000, while at 0.3 M NaCl (Figure 3b) free host factor migrates slower than aldolase with an apparent molecular weight of 120 000. Only at 1.0 M NaCl (data not shown) does host factor migrate close to the position of serum albumin (molecular weight 68 000). These data are consistent with an aggregate of three or more hexamers at 0.1 M NaCl and a mixture of one and two hexamers at 0.3 M NaCl.

RNA binding increases the tendency of host factor to aggregate. Complexes of (pA)$_{27}$ (molecular weight 8800) and host factor migrate more slowly than free host factors at both salt concentrations. The difference in molecular weight is considerably more than can be accounted for by the weight of the (pA)$_{27}$. For example, at 0.1 M NaCl free host factor migrates at 210 000 daltons, and the complex with (pA)$_{27}$ migrates at the void volume, indicating a molecular weight of >300 000. This effect of added RNA is presumably not due to the RNA acting as a "linker" between several smaller host factor aggregates, since under the conditions of these experiments—with a slight molar excess of (pA)$_{27}$ over host factor hexamers—the direct interaction of two or more hexamers with the same (pA)$_{27}$ molecule is not likely. Furthermore, very similar results are obtained with (pA)$_{15}$ where the oligomer is the same size as the RNA binding site of the

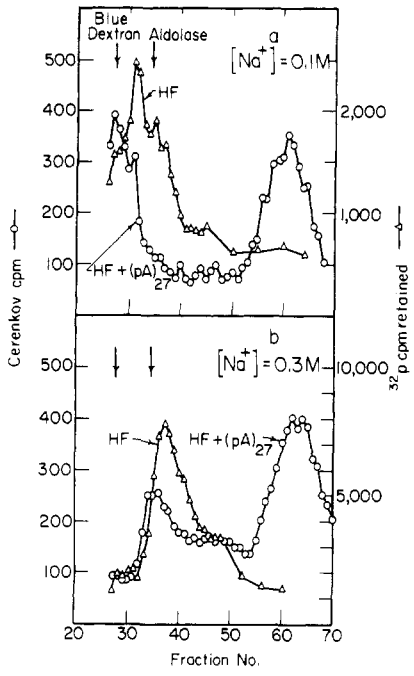

FIGURE 3: Gel filtration of host factor in the presence (O) or absence (Δ) of [5′-^{32}P](pA)$_{27}$. 76 pmol of host factor hexamers was chromatographed, and the activity was detected in individual fractions by a [5′-^{32}P](pA)$_{27}$ retention assay (Δ). Recovery was 7–10%. Alternately, 76 pmol of host factor was mixed with 110 pmol of [5′-^{32}P](pA)$_{27}$ [2.5 × 10^4 cpm in (a) 3.5 × 10^4 cpm in (b)]. Location in individual fractions (O) was detected by Cerenkov counting. Recovery was 10–15%.

hexamer (data not shown). Thus, the RNA–host factor complex aggregates somewhat better than the free host factor.

Discussion

In the previous paper (de Haseth & Uhlenbeck, 1980) the interaction of host factor with oligo(A)'s was investigated, allowing the determination of molecular parameters of the interaction. In this paper we demonstrate two additional important facts. First, below 0.3 M NaCl host factor hexamers aggregate to form multimers of two or more units without interfering with their ability to bind RNA. There is, in fact, some indication that host factor aggregation is greater in the presence of RNA. Second, Qβ RNA differs substantially from oligo(A) in its interaction with host factor. The stoichiometry of the complex at low ionic strength is six to twelve hexamers per Qβ RNA molecule [instead of one hexamer per oligo(A)]. Furthermore, the ionic strength dependence of the binding constant is much greater for Qβ RNA than oligo(A). At 0.1 M NaCl the binding of host factor to Qβ RNA is at least 10 times tighter than the binding to (pA)$_{27}$, while at 1.0 M NaCl it is at least 100 times weaker. Since the aggregation of host factor and its tight binding to Qβ RNA both show a similar ionic strength dependence, we propose that the two phenomena are related in the following simple model. Host factor hexamers can bind to several sites on Qβ RNA, each with an affinity considerably lower than that of oligo(A). Thus at high ionic strength, where no aggregation occurs, host factor binds less well to Qβ RNA than to oligo(A). However, at low ionic strengths the different members of an aggregate can simultaneously interact with different sites on Qβ RNA, lying close together in its tertiary structure, which results in a higher binding constant for the aggregate. Several aggregates would be able to bind to one Qβ RNA molecule in this fashion. Although this model is described in terms of the interaction of host factor with Qβ RNA, it should be applicable to the other RNAs studied (STNV RNA, 16S rRNA) as well.

It is not possible to determine from the available data whether the origin of the increased ionic strength dependence of K for natural RNAs is due to ionic protein–protein contacts formed between hexamers or due to the previously noted ionic component found for protein–RNA contacts. The relatively weak dependence of K on ionic strength seen for short oligo(A)'s binding to individual hexamers would be expected to increase substantially for the binding of several parts of a long RNA molecule to several hexamers in an aggregate. In the latter case, the ionic strength dependence of K will be intrinsically greater since the total number of charge interactions will be the sum of those formed with each hexamer (Record et al., 1976). Since we have not been able to characterize the nature of the aggregate at different ionic strengths, we cannot deduce the origin of the unexpectedly large ionic strength effects seen with natural RNAs.

The sites in Qβ RNA which bind the host factor hexamers remain unidentified. Senear & Steitz (1976) suggest that two oligonucleotides isolated by RNAse T$_1$ digestion of Qβ RNA and selection by host factor are binding sites for the protein. Consistent with our model invoking several weak binding sites for host factor on Qβ RNA, we find that the protein binds a T$_1$ digest of Qβ RNA with an affinity at least 3 orders of magnitude lower than the intact RNA. In addition, this clearly shows that the stretches of RNA which give rise to the two T$_1$ fragments cannot, in and of themselves, be responsible for the high affinity of host factor for Qβ RNA. The roles that these stretches of RNA play in the high-affinity binding of host factor, and in stimulation of RNA synthesis, remain unclear. Since oligo (A)'s of comparable lengths bind host factor considerably better than the T$_1$ oligomers, it is possible that the adenosine content of a sequence may be the major factor in its recognition by host factor. As has been shown by us here, and by Carmichael (1975), host factor interacts but weakly with homopolymers that do not contain adenosine residues. The smaller of the two T$_1$ oligomers has 9 adenines out of a total of 15 residues; the larger has 9 adenines out of 15 5′-terminal residues. Since host factor has a large oligomer binding site, the specific selection of the two T$_1$ fragments may simply be a consequence of the fact that they are the only A-rich oligomers in the T$_1$ digest (Billeter, 1978) of sufficient length to bind to the protein with a significant affinity. Thus, other sites on Qβ RNA, rich in A residues but also containing G residues, must be considered as possible binding sites for host factor. The selective binding of T$_1$ oligonucleotides of Qβ RNA by host factor is somewhat analogous to that seen with ribosomal protein S$_1$. The latter selects two oligonucleotides as well, one of which is selected by both proteins (Senear & Steitz, 1976; Goelz & Steitz, 1977). Binding appears to occur at the oligopyrimidine-specific site II on S$_1$ (Draper & von Hippel, 1978). Again the specificity of the selection is probably not due to the recognition of a particular sequence of the fragements, but rather of their length and base composition (high pyrimidine content) (Draper & von Hippel, 1978). The selection by S$_1$ of the 3′-terminal T$_1$ oligonucleotide of 16S rRNA (Dahlberg & Dahlberg, 1975) can be similarly explained (Draper & von Hippel, 1978; Lipecky et al., 1977). Both RNA–protein (Draper & von Hippel, 1979) and protein–protein (Laughtea & Moore, 1978) interactions are thought to be involved in the binding of S$_1$ to the 30S subunit, but their relative importance is unclear.

Clearly, caution must be exerted in the interpretation of fragment-selection data. Unless the fragment can be shown to rebind to the protein with the same affinity as the intact

RNA, a selection does not necessarily reflect relevant specificity between the protein and the RNA. In the present case, the base composition and length appear to be the determining factors in the binding of an oligonucleotide fragment to host factor. Optimal interaction of the protein to the intact RNA requires protein–protein contacts as well.

We have in the above discussion ascribed the large effect of ionic strength on the affinity between host factor and Qβ RNA to ion-dependent alterations in properties of the protein. It is possible, however, that a considerable rearrangement in the structure of Qβ RNA upon increasing the ionic strength from 0.1 to 1.0 M is responsible for the precipitous decrease in host factor binding affinity. At high salt concentration, more structure would form and the host factor binding sites would be covered. We believe that this explanation is unlikely, since magnesium ions, added to 10 mM, have an insignificant effect on the extent of competition by Qβ RNA in 0.1 M NaCl (data not shown). Magnesium ions are generally found to greatly increase RNA structure. Thus, unless 1.0 M NaCl has a specific effect on Qβ RNA structure that cannot be mimicked by $MgCl_2$, ion effects on the structure of the RNA cannot explain the weak binding of host factor to Qβ RNA at this salt concentration.

It is uncertain whether our model for the interaction of host factor with Qβ RNA can be applied to its interaction with poly(A). At high ionic strength, where aggregation of host factor does not occur, $[R_t]_{0.5}$ values for $(pA)_{27}$ and poly(A), when compared on a *per residue* basis, are similar. Thus, no additional free energy is obtained by contacts between adjacent protein molecules on poly(A), and we expect that host factor binds randomly (noncooperatively) along the RNA chain. At low ionic strength, one would expect extremely tight binding of host factor to poly(A), since both the strong association of host factor with A residues and the protein aggregation would contribute to the binding free energy. This does not seem to be the case, however. At low ionic strength, $[R_t]_{0.5}$ for poly(A) is only slightly less than that for $(pA)_{27}$. The interaction of host factor with poly(A) clearly merits further study.

The activity of host factor in stimulating Qβ replicase is consistent with our multisite binding model. Under the conditions of optimal stimulation, ~2 mol of host factor hexamer is required per mol of RNA (Franze de Fernandez et al., 1972; Carmichael et al., 1975; Kamen et al., 1972). Unless magnesium ions, present in the RNA synthesis reactions but not in our buffers, have a very specific effect, it is expected that the protein will be aggregated under the conditions at which it stimulates RNA synthesis. The requirement for an intact 3′ half of Qβ RNA for efficient host factor stimulated RNA synthesis by Qβ replicase (Schwyzer et al., 1972) might also be due to host factor interacting with regions of RNA which are distant from one another in the primary structure of Qβ RNA. Although host factor binds many RNAs and Qβ replicase will use many RNAs as a template, the stimulation of RNA synthesis catalyzed by the replicase is only seen with Qβ RNA. A subtle interplay of host factor and Qβ RNA replicase, binding specifically to Qβ RNA, must be required for the stimulation to occur.

The function of host factor in uninfected *E. coli* cells remains to be determined. Under the cellular ionic conditions, host factor hexamers will almost certainly form aggregates. These could bind to any poly(A) sequences within the cell, or to other RNA molecules, depending on the number and location of A-rich regions present on these. Host factor has been found to tightly interact with the 30S but not 50S ribosomal subunit (Carmichael et al., 1975; DuBow et al., 1977), but it is unclear whether this interaction has any bearing on its in vivo role. Thus, a broad potential exists for specific interactions involving host factor in both RNA metabolism and protein synthesis.

References

Alberts, B. M., & Frey, L. (1970) *Nature (London) 227*, 1313–1318.

Billeter, M. A. (1978) *J. Biol. Chem. 253*, 8381–8389.

Blumenthal, T., & Carmichael, G. G. (1979) *Annu. Rev. Biochem. 48*, 525–548.

Boedtker, H., & Gesteland, R. F. (1975) in *RNA Phages* (Zinder, N. D., Ed.) pp 1–28, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Carmichael, G. G. (1975) *J. Biol. Chem. 250*, 6160–6167.

Carmichael, G. G., Weber, K., Niveleau, A., & Wahba, A. J. (1975) *J. Biol. Chem. 250*, 3607–3612.

Dahlberg, A. E., & Dahlberg, J. E. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 2940–2944.

de Haseth, P. L., & Uhlenbeck, O. C. (1980) *Biochemistry* (preceding paper in this issue).

Draper, D. E., & von Hippel, P. H. (1978) *J. Mol. Biol. 122*, 339–360.

Draper, D. E., & von Hippel, P. H. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 1040–1044.

DuBow, M. S., Ryan, T., Young, R. A., & Blumenthal, T. (1977) *Mol. Gen. Genet. 153*, 39–43.

Franze de Fernandez, M. T., Hayward, W. S., & August, J. T. (1972) *J. Biol. Chem. 247*, 824–831.

Goelz, S., & Steitz, J. A. (1977) *J. Biol. Chem. 252*, 5177–5179.

Janik, B. (1971) *Physicochemical Characteristics of Oligonucleotides and Polynucleotides*, IFI/Plenum Press, New York.

Kamen, R., Kondo, M. Römer, W., & Weissmann, C. (1972) *Eur. J. Biochem. 31*, 44–51.

Kurland, C. G. (1960) *J. Mol. Biol. 2*, 83–91.

Laughrea, M., & Moore, P. B. (1978) *J. Mol. Biol. 122*, 109–112.

Lipecky, R., Kohlschein, J., & Gassen, H. G. (1977) *Nucleic Acids Res. 4*, 3627–3642.

McGhee, J. D., & von Hippel, P. H. (1974) *J. Mol. Biol. 86*, 469–489.

Record, M. T., Lohman, T. M., & de Haseth, P. L. (1976) *J. Mol. Biol. 107*, 145–159.

Reichmann, M. E. (1964) *Proc. Natl. Acad. Sci. U.S.A. 52*, 1009–1017.

Ruyechan, W. T., & Wetmur, J. G. (1975) *Biochemistry 14*, 5529–5534.

Schwyzer, M., Billeter, M. A., & Weissmann, C. (1972) *Experientia 28*, 750.

Senear, A. W., & Steitz, J. A. (1976) *J. Biol. Chem. 251*, 1902–1912.